THE ROLE OF HYDROGEN SULFIDE IN REGULATION OF MOUSE ATRIUM CONTRACTILITY

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Hydrogen sulfide (H_2S), along with nitric oxide and carbon monoxide, refers to endogenously synthesized gaseous molecules. H_2S has a number of effects in the cardiovascular system, both in normal and in various pathological conditions. In various tissues H_2S is synthesized from L-cysteine by enzymes cystathionine γ-lyase, cystathionine β-synthase and 3-merkaptosulftransferase. There are available data on the cardioprotective role of H_2S, expressed in the reducing of the myocardial damage in ischemia/ reperfusion in vitro and in vivo. In the myocardium of the frog H_2S has a negative inotropic effect, which is mediated by the activation of ATP-dependent K-channels (K (ATP) channel) and a decrease in the cAMP level in the cell. In a rat aorta smooth muscle the relaxing effect of H_2S is mediated by the activation of the potassium conductance. The aim of our work was to investigate the effects of exogenous and endogenous H_2S in mouse atrium, and to reveal the role of K-channels in the effects of H_2S.

Methods. The object of the study was the mouse Mus musculus. Experiments to determine the myocardium contraction were carried out using Biopac Systems, Inc. (USA). During the experiment, the preparation was perfused by Krebs solution. NaHS was used as H_2S donor, Also L-cysteine, β-cyano-L-alanine, tetraethylammonium (TEA), glibenclamide, diazoxide, L-NAME (Nitro-L-arginine methyl ester) were used (Sigma).

Results and discussion. To reveal the effects of exogenous H_2S on the myocardial contractility, NaHS was cumulatively applied in concentrations of 100, 200 and 300μM Application of NaHS at concentrations of 100, 200 and 300 μM resulted in a significant decrease in the force of contraction to the 15 minute to 91 ± 2% ($n = 14$, $p < 0.05$), 71 ± 4% ($n = 14$, $p < 0.05$) and 49 ± 4% ($n = 15$, $p < 0.05$), respectively. To identify the possible endogenous H_2S synthesis in the atria of the mouse L-cysteine – substrate and - β-cyano-L-alanine – inhibitor of H_2S synthesis were used. L-Cysteine at concentrations of 1, 10, 50 μM resulted in a significant decrease in force of the contraction to 95 ± 1% ($n = 7$, $p < 0.05$), 89 ± 1% ($n = 5$, $p < 0.05$), 87 ± 2% ($n = 5$, $p < 0.05$), respectively, whereas the use of higher concentrations of L-cysteine (2 mM) resulted in increased force of contraction, which was 121±4% ($n=3$, $p<0.05$). Possibly, L-cysteine at low concentrations serves as a substrate of H_2S synthesis, but in high concentrations inhibits the H_2S synthesis through a mechanism of the substrate-enzyme inhibiting. Application of β-cyano-L-alanine in a concentration of 1 mM led to a significant increase in myocardial force up to 112 ± 5% ($n = 5$, $p < 0.05$). Thus, the substrate of H_2S synthesis - L-cysteine caused a reduction in the amplitude of myocardial contractions like the donor of H_2S - NaHS, whereas the blocker of cystathionine γ-lyase had the opposite effect - the increase in the amplitude of contraction. Our results suggest that, in mouse myocardium, as with other classes of vertebrates, there is a system of H_2S synthesis, which involves its modulatory effect on the myocardium. One of the mechanisms of the muscle relaxation is an activating of the potassium conductance. Glibenclamide, an inhibitor of K(ATP)-channels, in the concentration of 50 μM increased the contraction to 121 ± 5% ($n = 6$, $p < 0.05$) from the control level, which is apparently due to the inhibition of K(ATP)-channels, membrane depolarization and increased Cainward currents. On the background of glibenclamide NaHS, at a concentration of 100, 200 and 300μM, did not result in a significant reduction decrease force, which amounted to 99 ± 1% ($n = 6$, $p > 0.05$), and which was significantly different from control values. In concentration of the effect NaHS was preserved and was 83 ± 2% ($n = 6$, $p < 0.05$), 47 ± 7% ($n = 6$, $p < 0.05$), respectively. Diazoxide was used as an activator of K(ATP)-channels at a concentration of 100μM, which application led to a significant decrease in the force of contraction to 96 ± 2% ($n = 7$, $p > 0.05$). On a background of diazoxide the effect of an application of NaHS in concentrations of 100, 200, 300 μM was 81 ± 4% ($n = 7$, $p < 0.05$), 63 ± 9% ($n = 5$, $p < 0.05$), 42 ± 10% ($n = 4$, $p < 0.05$). To test the involvement of NO-system we used L-NAME that blocks the synthesis of NO. Application of L-NAME in concentration 300 μM does not lead to a significant change in the force of contraction, which was 102 ± 3% ($n = 12$, $p > 0.05$). Application of NaHS in concentrations of 100, 200 μM induces the same decrease in the amplitude of contractions as in control (95 ± 1% ($n = 12$, $p < 0.05$) and 81 ± 4% ($n = 10$, $p < 0.05$)), whereas in concentration 300 μM the decrease was significantly less than in control conditions (73 ± 5% ($n = 10$, $p < 0.05$)).

Thus, our results suggest that, in mouse myocardium, as with other classes of vertebrates, there is a system of H_2S synthesis, which has a modulating effect on a cardiac contractile function. It was shown that the activation of K(ATP)-channels is mediated by effects NaHS in low concentrations, whereas at higher concentrations, apparently may be involved the NO synthesis.

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MECHANISMS OF NO-MEDIATED MODULATION OF NON-QUANTAL ACETYLCHOLINE RELEASE AT THE MAMMALIAN NEUROMUSCULAR JUNCTION

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Identification of neuronal isoform of the enzyme nitric oxide (NO) synthase (type I) in the postsynaptic region of mammalian muscle fiber suggested its involvement in the neuromuscular transmission. NO molecules are short-lived free radicals able to penetrate through cell membranes. Because of this, they have been considered as retrograde signal molecules in peripheral synapses. As was shown earlier, that NO at mammalian neuromuscular junctions (unlike amphibian synapses) does not affect processes of the quantal acetylcholine release, but significantly inhibits the intensity of the non-quantal mediator secretion. Since the majority of data indicate that the tonic neurotransmitter release is one of neurotrophic control factors, the study of mechanisms of the regulation (including NO-mediated) of this type of neurosecretion is quite topical.

Using electrophysiological methods we have revealed the following: i) constitutive activity of NO synthase affects the level of the intensity of the non-quantal acetylcholine release from rat motor nerve endings; ii) the enhancement of NO molecules synthesis can be initiated by the activation of both muscarinic M_1 cholinoreceptors and glutamate N-methyl-D-aspartate (NMDA) receptors; iii) key moments in mechanisms of muscarine- and glutamate-induced inhibition of the non-quantal acetylcholine release are the entry of Ca^{2+} from the extracellular medium, enhancement of NO molecules synthesis, the transsynaptic (retrograde) action of NO and the activation of the soluble guanylyl cyclase in nerve ending.

The fact that the extracellular calcium-dependent mechanism of NO-mediated modulation of the non-quantal acetylcholine secretion is able to run via the activation of both NMDA receptors